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Chemical Properties of Lipopolysaccharides (LPS) isolated from *Vibrio anguillarum* PT 514

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Vibrio anguillarum, one of the causative agents of fish vibriosis, is serologically and biochemically divided into three groups (A, B and C). The chemical composition and molecular architecture of lipopolysaccharide (LPS) isolated from *V. anguillarum* PT 514, which belongs to serogroup B, were investigated. The LPS contained glucose (Glc), fructose (Fru), L-glycero-D-mannoheptose (L-D Hep), glucosamine (GlcN) and 4-amino-4,6-dideoxyglucose as sugar constituents in molar ratios of 8.9 : 0.7 : 3.0 : 1.1 : 1.6. Sephadex G-50 gel-chromatography of a degraded polysaccharide fraction separated from the LPS by 5 % acetic acid hydrolysis suggested that the O-specific polysaccharide region consists of, in average, as much as 29 moles of Glc per 3 moles of L-D Hep, while the core polysaccharide contains at least Glc, L-D Hep and GlcN in molar ratios of 3.2 : 3.0 : 0.2. Fru and 4-amino-4,6-dideoxyglucose components were released from LPS on weak-acid hydrolysis, indicating that PT 514 LPS is distinguishable from those of *Vibrio anguillarum* belonging to the other serogroups. 2-Keto-3-deoxyoctonate (KDO), a common sugar constituent of gram-negative bacterial LPS, was not detected by Weissbach's color reaction under the conventional hydrolysis condition, but *O*-phosphoryl KDO was found in the strong-acid hydrolysate (4 M HCl, 100 C, 45 min). This substance was identical, at least in high-voltage paper electrophoresis, to 5-*O*-phosphoryl KDO. Lipid A isolated from the LPS contained $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:1}$, 3-$OHC_{12:0}$ and 3-$OHC_{14:0}$. The LPS showed endotoxic activity in limulus lysate assay to the same extent as those of *V. cholerae* NIH 41 (Ogawa) and 569B (Inaba).

V. anguillarum was first named and reported by Bergman[1] as the pathogen of vibriosis which is an infectious disease of seawater and of freshwater fish caused by Vibrio genus bacteria . Cases due to the present bacterium are discovered widely everywhere across the world. In Japan, the disease is often found in ayu fish, eels, rainbow trouts and such, and has become a problem for fish farming. Taxonomic and serologic studies of *V. anguillarum* have been conducted for a long time. Nybelin [17] divided *V. anguillarum* into the two A and B types on the basis of indole productivity and of cane sugar and mannitol dissolubility. Later, Smith [22] added the type C. In Japan, Ueda et al divided the bacterium into 3 types according to the biological and biochemical characteristics of the bacteria isolated from diseased fish, that is, into a type originating from freshwater, another type originating from seawater and one more intermediary type [12]. They also elucidated the serological specificities of the particular bacteria through sedimentation reactions in gel, and demonstrated that the division into three types was sound [13]. Meanwhile, Mibuchi et al made detailed epidemiological examinations of the vibriosis of ayu fry originating from the Hamana lake, and

divided the serological types of *V. anguillarum* into the three A, B, C types. They also reported on the correlation between these serotypes and the capture time as well as the sensitivity to drugs of the diseased fish [15,16]. We extracted and purified lipopolysaccharides (LPS) from strains of these three serotypes and researched the correlation between the chemical types based on sugar compositions and the serotypes. The results demonstrate that the LPS sugar compositions correspond to the serotypes of the bacteria, and that they have their own particular patterns [4]. We also examined the biological activities of these LPS such as mitogenicity, adjuvanticity or resistance to tumors. We noted that the LPS of the *V. anguillarum* PT 514 strain belonging to serotype B showed a remarkable antitumor action [20,21]. In the present study, we eliminated the lipid A fraction through acetic acid heating of the LPS, performed the Sephadex G-50 gel chromatography of the obtained total degraded polysaccharides - DPS [3], as well as the chemical analysis of the sugar compositions of the particular eluted fractions in order to find the chemical properties, and more particularly the molecular structure of the LPS of the PT 514 strain. While elucidating the fatty acid compositions of the

lipid A which is the main agent of the LPS endotoxicity, we also examined the endotoxic activity of the LPS.

Materials and Method

We used *V. anguillarum* PT 514 strains obtained from Dr. Kitao of the Miyazaki University. We performed shaking culture of the bacterial strains for 16 h in 2 % table salt-supplemented bouillon media (pH 7.4) at 27 °C, gathered the bacteria after heating for 20 min at 120 °C, washed three times with distilled water and used the bacteria as acetone-dried samples. We extracted the LPS from the acetone-dried bacteria by the phenol-water method of Westphal et al [26], performed repeated centrifugation (105,000 x g, 3 hours, 6 times) and purified by RNase treatment. We prepared the DPS by evaporating to dryness the centrifugation supernatant after a 100 °C, 3-hour hydrolysis of the LPS with 5 % acetic acid. We prepared the lipid A by washing the centrifugal precipitation 3 times with distilled water. We converted the fructose (Fru)-freed neutral sugars to alditol-acetone

derivatives following the hydrolysis of the LPS conducted at 120 °C for 2 hours with 2N trifluoroacetic acid. Also, converting Fru to 0-acetyl-0-methyloxime derivatives after performing for 2 hours at 100 °C with 5 % acetic acid the hydrolysis of the LPS, we performed the qualitative and quantitative analyses by gas chromatography, using a 3 % ECNSS-M column. We converted the amino saccharides to N-acetyl-alditolacetate derivatives after performing the hydrolysis of the LPS for 8 hours at 100 °C with 4N hydrochloric acid, and carried out the qualitative and quantitative analyses, using a TAB-SORB column. We performed the colorimetric quantitative determination of the heptose (Hep) by the Osborn method [18], and of the 2-keto-3-deoxy-octonate (KDO) by the periodic acid-thiobarbituric acid reaction of Weissbach et al [25]. We converted the fatty acids to methylester derivatives after hydrolyzing at 100 °C for 4 hours with 4N hydrochloric acid and, performed the qualitative and quantitative analyses by gas chromatography, using a 25 % DEGS column. We performed the ester exchange reaction of the lipid A for 10 hours at 37 °C, using 0.25 N sodium methylate in anhydrous methanol [19]. For the gel chromatography of DPS, we used a Sephadex G-50 column (2.6 x 100 cm), and eluted at a flux of 39 mL/h with a pyridine-acetic acid buffer solution (pyridine : acetic acid : water = 10 : 4 : 1,000, v/v/v). We fractionated the eluate into 3mL aliquots, and used as detector a differential refractometer (Atto SF 1107, Ato* [1] Co.). For the high-voltage filter paper electrophoresis, we used a pyridine : acetic acid : water (10 : 4 : 86, v/v/v, pH 5.3) solvent system on Whatman No 1 filter paper, and performed electrophoresis for 50 min at 50 v/cm. To detect the KDO on the filter paper, we used a thiobarbituric acid reagent [24]. We measured the endotoxic activity of the LPS by the Limulus (LAL) tests, and by the colorimetric method using synthetic substrates (Biotic-Seikagaku Industries).

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* Translator's note : as transliterated

Results

The sugar composition of the LPD

The upper part of Table 1 shows in terms of weigth % and relative molar ratio per three moles of heptose the sugar compositions of the LPS of the *V. anguillarum* PT 514 strain . The detected LPS structural sugars were glucose (Glc), Fructose (Fru), L-glycero-D-mannoheptose (L-D Hep), glucosamine (GlcN), and 4-amino-4, 6-dideoxyglucose (4-amino-4,6-dideoxy-Glc). Of these, Glc, L-D Hep, GlcN are found not only in the LPS of the other *V. anguillarum* serotype strains, but in the LPS of all the Vibrio family bacteria which we have examined so far [5]. Meanwhile, Fru and 4-amino-4,6-dideoxy-Glc were within the *V.anguillarum* LPS, specific sugars present only in the LPS of the serotype B strain which contains the PT 514 strain [21]. Among these, Fru is known to be a structural sugar of the LPS of *V. cholerae* (and more particularly of the 01 type *V. cholerae*) which is a Vibrio genus bacterium [10]. Fru is rarely contained in LPS other than those of *V. cholerae*. Furthermore, in the bacteria of the Vibrio family, 4-amino-4,6-dideoxy-Glc is reported to be found in some *V. parahaemolyticus* 08 and 09 as well as in some *V. alginolyticus* [5].

The presence of KDO phosphorylated derivatives detected under strong-acid hydrolysis conditions

No KDO was detected (0.02 N sulfuric acid, 100 °C, 20 min) under ordinary hydrolysis conditions by the method of Weissbach. When however hydrolyzing the LPS with strong acid (4 N hydrochloric acid, 100 °C, 45 min), the thiobarbituric acid reaction-positive substance X was detected in the hydrolyzate, and the peak absorption wavelength of the reaction solution was 549 nm as in the case of KDO. However, as Fig. 1 shows, this X indicates in high-voltage filter paper electrophoresis a mobility clearly different from

that of KDO (relative to KDO, the shift degree is $R_{KDO} = 1.54$), and it is suggested that this substance is identical to the TBA reaction-positive substance X_1[11] contained in the strong-acid hydrolysates of the LPS of all the Vibrio family bacteria. Moreover, the mobility of this X matched the mobility of the phosphoryl-KDO [2] isolated from the strong-acid hydrolysates of the *Bordetella pertussis* (Phase I bacterium) LPS. Furthermore, we treated with alkaline phosphatase (ammonium carbonate buffer solution, pH 9.6, 37 °C, 16 h) the particular phosphoryl-KDO originated from the X of the *V. anguillarum* LPS and from the *B. pertussis* LPS, and performed afterwards high-voltage filter paper electrophoresis. As a result, we lost the particular spots corresponding respectively to the X and to the phosphoryl-KDO, and there appeared instead new spots having the same mobility as that of KDO. The above results suggest that the X originated from *V. anguillarum* PT 514 strain is similarly to the TBA reaction-positive substances found in the other LPS of the Vibrio family bacteria, a phosphorylated derivative of KDO.

Gel chromatography of the DPS

Generally the DPS obtained through the acetic acid-hydrolysis (1 p. 808 %,100°C,1.5-2.5 hours) of the S-type LPS of gram-negative bacteria such as Salmonella or Bacillus coli are divided into three fractions by Sephadex G-50 gel chromatography [27]. There is a first elution of the total polysaccharide chain portion of the LPS in which the O antigen polysaccharide side chains are bonded with core polysaccharides in the void volume (Frc. I). Following this, the core polysaccharides elute in the imbibed volume (Frc. III). Thereafter, and immediately ahead of the bed volume is in turn eluted the fraction containing the inorganic salts and the free KDO (Frc. IV). The DPS prepared by the weak-acid hydrolysis of the LPS of the *V. anguillarum* PT 514 strain are divided into four fractions by gel filtration, and exhibit an aspect fairly different from the DPS of the Salmonella and Bacillus coli, or of Cholera bacteria which are divided into three fractions, or of *V. parahaemolyticus* or *V. vulnificus* which are divided into the sole two Frc. III and Frc. IV. This means that, as Fig. 2 shows, Frc. I was eluted first in

the void volume, to be followed a little later by a small volume of Frc. II, and then later by large volumes of Frc. III and Frc. IV.

The various fractions obtained by the gel chromatography of DPS and the sugar composition of Lipid A

Table 1 shows the various fractions (Frc. I-IV) obtained through the gel chromatography of the DPS as well as the chemical composition of the lipid A. A large volume of about 29 moles of Glc was detected per 3 moles of L-D Hep in Frc. I, and glucan was expected to be admixed. So, using for the PT 514 strain the simple separation method extracting directly the sole LPS polysaccharide portion from the bacteria by trichloroacetic acid-based deproteinization, and by acetone fractionation [8], we performed the sugar analysis of the obtained polysaccharide fraction. As a result, the L-D Hep and Glc molar ratio indicated about 3:21, and a high content of Glc was detected like in Frc.I of the DPS. The possibility of glucan admixture into the LPS was denied. Frc. II and Frc. III contained Hep with a Glc and L-D molar ratio of about 3:3, as well as a small amount of GlcN. A large volume of Fru and of 4-amino-4,6-dideoxy-Glc as well as a small volume of Glc in free condition were detected in Frc. IV. One observed also a large volume of inorganic phosphorus in this fraction. Meanwhile, the only constitutive sugar contained in the lipid A was GlcN, and the structural molar ratio vs. this phosphoric acid was about 1:1. The volume of GlcN contents in the DPS fraction was extremely small, and it was suggested that GlcN forms the backbone of the lipid A in the PT 514 LPS.

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The fatty acid compositions of lipid A

Table 2 shows the lipid acid composition of the lipid A prepared from the LPS of the *V. anguillarum* PTS 514 strain. The present lipid is mainly constituted of myristic acid ($C_{14:0}$), 3-hydroxylauric acid (3-OH $C_{12:0}$), and 3-hydroxymyristic acid (3-OH $C_{14:0}$). One detected also a small amount of lauric acid ($C_{12:0}$), palmitic acid ($C_{16:0}$), palmitooleic acid ($C_{16:1}$) and oleic acid ($C_{18:1}$). As a result of sodium methylate-based ester exchange

reaction, it was shown that among these fatty acids, only 3-OH $C_{14:0}$ was an amide-bound fatty acid.

p. 809

The endotoxic activity of the LPS

In the numerous endotoxic activities of the LPS, lipid A which is the fatty portion of the LPS molecule, and more especially its fatty acids play an important role. We measured the endotoxic activity of the LPS of the *V. anguillarum* PT 514 strain through endotoxin quantitative tests based on Limulus tests and colorimetry. For the control, we used LPS of *Escherichia coli* 0111 from the Sigma Co., and compared with the LPS endotoxic activities of *V. cholerae* NIH 41 (Ogawa) and 569 B (Inaba). Table 3 shows the endotoxin activities of the particular LPS, based on the Limulus tests, and Fig. 3 shows the results obtained by the endotoxin quantitative tests based on the colorimetric method. In both tests, the LPS of the *V. anguillarum* PT 514 strain showed lower activity values than the Ogawa-type cholera bacterium and about the same as those of the Inaba-type cholera bacterium. The endotoxic activity was higher than in the LPS of *E. coli* 0111 used as control. More particularly, the PT 514 LPS showed in the Limulus test an activity about 10 times stronger than that of the LPS of *E. coli* 0111, and it was shown that the LPS of the present bacterium have highly active endotoxins.

Discussion

The polysaccharide portion of the LPS is the center of 0 antigen activity in the gram-negative bacteria, and plays an important role in the determination of the serological specificity of the bacteria. The chemical structure of the O antigen specific polysaccharides corresponding to the O antigenic determinant factors regarding the O antigen determining this 0 specificity was elucidated recently with the LPS of numerous types such as *Salmonella, Shigella, Escherichia*. Special detailed examinations were performed for the *Salmonella* genus [14]. We too examined the LPS of *V. anguillarum* of the various A, B, C serotypes isolated from ayu fry collected

in the Hamana lake, and demonstrated the presence of sugars differing in the respective LPS [4,21]. Yet, the presence position, the mode of combination of these sugars in the LPS, or the chemical structure background of the LPS as a whole have not been clarified. This is why we performed now the gel chromatography of the DPS of the *V. anguillarum* PT 514 strain belonging to serotype B. p. 810

As a result, the DPS were divided into 4 fractions, and showed elution patterns analogous to that of the *Plesiomonas shigelloides* DPS 6). It was shown that the molecular weight of the DPS of the PT 514 strain was higher than those of *V. cholerae* and *V.parahaemolyticus*. A large volume of Glc was detected from the Frc. I eluted in the void volume, and there seemed to be an admixture of glucan produced by the bacteria. However, as we reexamined the PT 514 strain by the simple separation method which we developed, extracting only the LPS polysaccharide portion, we detected indeed large contents of Glc in the samples. It is consequently estimated that the large volume of Glc found in the LPS of the PT 514 strain is not caused by the admixture of glucan but originates from the LPS themselves, and that the polysaccharide side chains of this bacterium are made of polymers whose main component is Glc. Meanwhile, it was shown that the core part was made from an outer core part containing L-D Hep and Glc in an approximate molar ratio of 3:3, and of an inner core part containing phosphorylated KDO. Since Fru and 4-amino-4, 6-dideoxy-Glc which are the specific sugar of *V. anguillarum* of the serotype B are eluted jointly as free sugars in the monosaccharide fraction (Frc. IV), it seems that both are present in the LPS in a bonding mode extremely weak with regard to weak acids. Of these, Fru is specifically detected within the Vibrio family bacteria from the LPS of 01 *V. cholerae* 10) and one knows that this Fru is also easily separated by acetic acid superheating treatment. We have already reported on the correlation between the bonding mode and the 0 antigen specifity in the LPS 7), and it seems that the Fru in the LPS of *V. anguillarum* also, similarly to that in 01 *V. cholerae*, could be present through a ketoside-bond in a branched manner inside the polysaccharide part of the LPS. Meanwhile, 4-amino-4, 6-dideoxy-Glc is proven to be present in the LPS of *V. parahaemolyticus* 09 23). However, as this sugar is detected in a bonded mode in the core polysaccharide fraction (Frc. III), it seems to have a bonding mode clearly different from that observed in *V. anguillarum*, which is

detected as free sugar in Frc. IV. Consequently, the 4-amino-4, 6-dideoxy-Glc originated from *V. anguillarum* was, similarly to Fru, estimated to be easily isolated through acid hydrolysis in the LPS polysaccharide portion and to be bonded in branched manner. The detailed bonding modes are not quite clear in the polysaccharide portions of both free sugars. Yet, as they are specifically detected from the LPS of the B-type *V. anguillarum*, it would seem that both sugars are in some way involved in the serotype appearances.

Generally, the KDO present in the inner core of the gram-negative bacteria are in the case ofthe LPS of the *V. anguillarum* PT 514 strain, and unlike for the Salmonella or Bacillus coli bacteria, not detected by the weak-acid hydrolysis-based Weissbach method 25), but were first detected in the form of bond with phosphoric acid through strong-acid hydrolysis. Consequently, it was estimated that in the LPS of this bacterium, the outer core and lipid A are combined by the phosphorylated KDO. The presence of phosphorylated KDO is, with a few exceptions, one of the chemical characteristics common to almost all LPS in the Vibrio family bacteria, and it seems that this could be an important index for the establishment of the Vibrio family bacterial chemotaxonomy.

The LPS exhibit an extremely colourful bioactivity. Their immunological activity as O antigen (bacterium surface antigen) is a remarkable fact involving the participation of the polysaccharide part. The lipid A part participates in the appearance of endotoxic activities such as lethality and pyrogenicity. In the endotoxin quantification tests based on the Limulus test and on the colorimetric method, the LPS of the *V. anguillarum* PT 514 strain were compared to those of the Ogawa-type *V. cholerae*, and revealed a lower endotoxic activity. The level was the same as in the Inaba-type *V. cholerae*, and higher than in the *E. coli* 0111 LPS. The endotoxic activities were remarkably decreased when treating the PT 514 strain LPS with alkali. It seems therefore that the appearance of endotoxic activity of the LPS of the present bacterium requires the presence of fatty acids in which both the esters and amides remain intact in their combination with the lipid A.

As shown above, the study of molecular structure centered on the LPS sugar composition supporting the appearance of the O antigen specificity is indispensable to explain the antigen factors specific to the species. The

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differences in sugar compositions are strong confirmations of the serotype determinations. At the present time, the serotypes of *V. anguillarum* are not limited to A, B, C. More diversification seems to be coming. Kitao 9) et al are reporting the new D, E, F serotypes. The microstructures of the LPS of these bacteria should of course be different. One is looking forward to further research in the structures.

Acknowledgment

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Explanations of Graphs and Figures

Table 1 The fractions and the Lipid A chemical compositions (%, w/w) obtained through the gel chromatography of LPS, DPS prepared from *V. anguillarum* PT 514

4) total volume of lipid in the sample
5) molar ratio per 3.0 moles of L-D Hep
6) ---- or lower

a) yield rate from acetone-dried bacteria

b) yield rate from LPS

Figure 1 The thiobarbituric acid reaction-positive substances detected in the strong-acid hydrolysates of the LPS of *V. anguillarum* PT 514, *V. cholerae* O3 and *V. parahaemolyticus* O2 and the high-voltage filter paper electrophoresis of phosphorylated KDO obtained from the LPS of *B. pertussis*

electrophoresis : 50 v/ cm, 50 min electrophoresis, buffer solution : pyridine/acetic acid/water (10:4:86, v/v/v), development reagent : periodic acid, thiobarbituric acid

Figure 2 Sephadex G-50 gel chromatography of the DPS fraction isolated from *V. anguillarum* PT 514 LPS

Column : Sephadex G-50, 2.6 x 100 cm. Eluate : pyridine/acetic acid/water, 10:4:1000 (v/v/v). Elution rate : 39 mL/h. Detector : differential refractometer

a. absorption

Table 2 Fatty acid composition of the Lipid A separated from the LPS of *V. anguillarum* PT 514

1) weight of detected fatty acid represents 100 %

2) molar ratio per 2.0 moles of 3-OH $C_{14:0}$

Table 3 Compared Limulus test-based endotoxic activities of the LPS of *V. anguillarum* PT 514 and those of the 01 *V.cholerae* and *E. coli* 0111

Figure 3 Compared colorimetric method (Hairodikku* tests)-based endotoxic activities of the LPS of *V. anguillarum* PT 514, and of those of 01 *V. cholerae* and *E. coli* 0111

a. absorption

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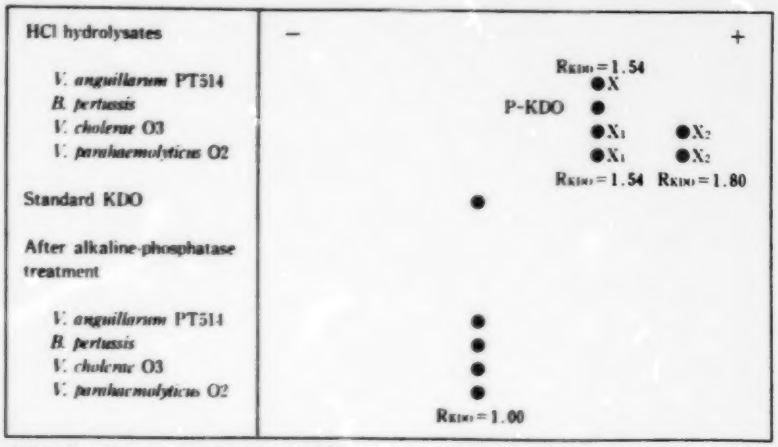
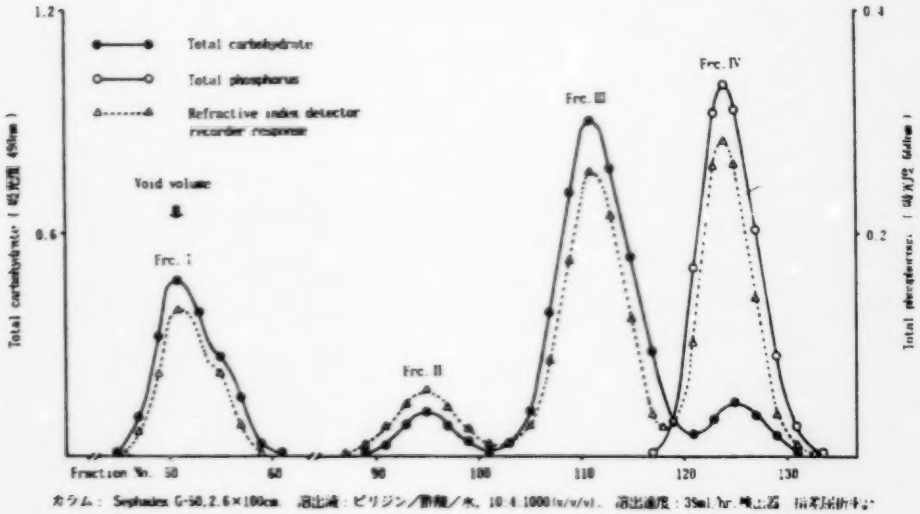
* Translator's note : as transliterated; meaning uncertain



電気泳動：50v/cm, 50分間泳動，緩衝液：ピリジン／酢酸／水（10：4：86，v v v），
発色試薬：過ヨウ素酸・チオバルビツール酸．

図1　*V. anguillarum* PT514, *V. cholerae* O3 と *V. parahaemolyticus* O2 LPS の強酸加水分解物中に検出されるチオバルビツール酸反応陽性物質と *B. pertussis* LPS より得られたリン酸化 KDO の高圧連続電気泳動



カラム：Sephadex G-50, 2.6×100cm　溶出液：ピリジン／酢酸／水，10:4:1000(v/v/v)，溶出速度：35ml/hr，噴出法　画分採取量：

図2　*V. anguillarum* PT514 LPS より分離した DPS 画分の Sephadex G-50 ゲルクロマトグラフィー

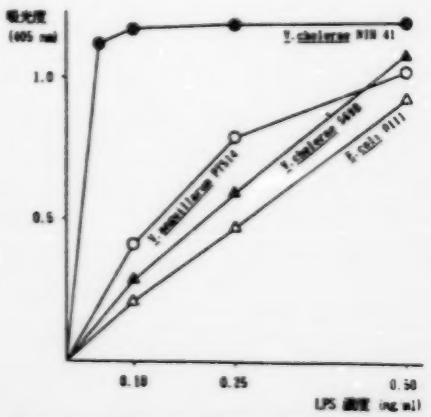


図3　*V. anguillarum* PT514 LPS と O1 *V. cholerae* および *E. coli* O111 LPS の比色法（ハイロティック試験）による内毒素活性の比較

表1　*V. anguillarum* PT514から調製したLPS, DPSのゲルクロマトグラフィーにより得られた各画分およびLipid Aの化学組成〔%, w/w〕

	Yield	Glc	Fru	L-D[1] Hep	GlcN	4 amino 4.6[2] d Glc	Total[3] P	Total[4] Lipid
LPS	1.7[a]	37.3 (8.9)[5]	2.8 (0.7)	14.7 (3.0)	4.6 (1.1)	6.2 (1.6	1.8	17.3
DPS								
Frc. I	14.5[b]	36.4 (29.0)	— (—)	4.4 (3.0)	— (—)	— (—)	—	—
Frc. II	5.2[b]	20.3 (2.7)	— (—)	26.3 (3.0)	1.7 (0.2)	— (—)	—	—
Frc. III	21.8[b]	36.3 (3.2)	— (—)	40.3 (3.0)	2.1 (0.2)	— (—)	—	—
Frc. IV	11.4[b]	1.8	8.1	—	tr[6]	17.4	9.9	—
Lipid A	32.3[b]	tr	—	—	10.1	—	2.3	60.0

[1] L-D Hep：L-glycero-D-mannoheptose,
[2] 4-amino-4,6-d-Glc：4-amino-4,6-dideoxyglucose,
[3] Total P：Total phosphorous,
[4] 試料中の全脂質含量,
[5] L-D Hep 3.0モルに対するモル比,
[6] tr：trace amount, 0.1%以下
[a] アセトン乾燥菌体からの収率,
[b] LPSからの収率,

表2　*V. anguillarum* PT514 LPSから分離したLipid Aの脂肪酸組成

Fatty acid	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:1}$	3-OH $C_{12:0}$	3-OH $C_{14:0}$
(%, w/w)[1]	3.6	28.2	2.9	4.0	2.2	23.6	35.5
(molar ratio)[2]	(0.2)	(1.7)	(0.2)	(0.2)	(0.1)	(1.5)	(2.0)

[1] 検出された脂肪酸の重量を100%とした, [2] 3-OH $C_{14:0}$ 2.0モルに対するモル比。

表3　*V. anguillarum* PT514 LPSとO1 *V. cholerae* および *E. coli* O111 LPSのリムルステストによる内毒素活性の比較

	Concentration of LPS (ng/ml)				
	0.001	0.005	0.01	0.05	0.10
V. cholerae NIH 41 (Ogawa)	+	+	+	+	+
V. anguillarum PT 514	−	±	+	+	+
V. cholerae 569 B (Inaba)	−	±	+	+	+
E. coli O111 Reference	−	−	−	±	+

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